

Protein Concentration of Excretory-Secretory and Somatic Antigen of *Paramphistomum* spp.

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ABSTRACT

This study was aimed at finding out protein profile of excretory-secretory and somatic antigen of *Paramphistomum* spp. As many as 60 *Paramphistomum* spp. taken from cattle in Banda Aceh Municipality Slaughterhouse were placed into 80 ml Roswell Park Memorial Institute (RPMI) 1640 and incubated for 12, 24, 48, and 72 hours. Excretory-secretory as well as somatic antigen was isolated and the concentration of protein was determined using Bradford method, and calibration curve was conducted using Bovine Serum Albumin as standard. To find out the molecular weight of protein, SDS-PAGE method was employed. The linear regression of calibration curve was $y = 0.451x + 0.651$. The highest concentrations of excretory-secretory antigen protein obtained was 235 ppm, and the somatic protein was 261 ppm. Weight of protein molecules was ranging from 29-130 kDa.

Keywords: excretory-secretory antigen, *Paramphistomum* spp., SDS-PAGE, protein profile, antigen isolation.

1. INTRODUCTION

The trematode parasite of *Paramphistomum* spp. are causative agent for paramphistomiasis in several hosts; cattle, goat, buffalo and sheep [1]. *Paramphistomum* spp. excysts in intestine and then juvenile flukes migrate to reticulum until reach adult stage [2-3]. This fluke will migrate when the temperature is around 25-30 °C with humidity around 85% [5]. Adult *Paramphistomum* spp. has 5-13 mm length and 2-5 mm width [5], while the egg has 113-175 µm length and 73-100 µm width with light yellowish and transparent appearance [3, 6]. Living in rumen and abomasum of ruminants, this trematode causes retarded growth and significant economic loss. There was a report [7], stated a loss of US 2, 5 billion annually only in Indonesia. To cope with the pathogenicity and potential loss *Paramphistomum* spp. infection, appropriate control measures must be taken. Accurate diagnosis is substantial in determining the measures to be taken to eradicate this parasitic disease. On top of this, the search of specific antigen released by *Paramphistomum* spp. is also an alternative solution to overcome the disease. The development of immunology technique and molecular biology are standard tools in antigen and protein diagnosis [8-9], where the antigen is

used as the target of antibody [2, 3, 10]. Excretory-secretory (ES) antigen can be found as protein resulting from metabolic processes when fluke is established in the host body [8, 9, 11]. Besides ES antigen, fluke also contain somatic antigen and superficial antigen which also can be recognized by the host [4, 12]. However, ES antigen has a better capacity in triggering immune response and it is assumed that it has more protective effect in the immune system [2, 9].

Excretory-secretory is an antigen produced by metabolism in helminths, which can stimulate host immune response [13]. Besides ES antigen, there are also somatic antigen and superficial antigen which could be recognized by the host to stimulate immune response. However, ES antigen are more potential compared to other antigen in provoking immune system and therefore are more beneficial as immunodiagnostic tool [14]. In this study, we would like to elaborate the identification and characterization of ES antigen, and further determine the concentration of ES antigen protein from *Paramphistomum* spp. Protein profile was also presented and analyzed using Bradford assay and Sodium Dodecyl Sulphate Poly-Acrylamide Gel Electrophoresis (SDS-PAGE).

2. MATERIALS AND METHODS

This experiment was conducted to isolate and identify ES antigen protein from *Paramphistomum* spp. using Bradford assay. Protein concentration was determined based on calibration curve using Bovine Serum Albumin (BSA). Molecular weight of protein was visualized with SDS-PAGE.

2.1. Isolation of ES Antigen and Somatic Protein of *Paramphistomum* spp.

Paramphistomum spp. flukes were obtained from rumen and abomasum of cattle from slaughterhouse in Banda Aceh. As many as 60 flukes were rinsed six times with phosphate buffer saline (PBS), pH 7.3, and then incubated in Roswell Park Memorial Institute (RPMI) 1640 media at 37 °C [15]. The protein isolated was differentiate between supernatant which contain ES antigen, and pellet for somatic antigen. The incubation time was 12 hours, 24 hours, 48 hours, and 72 hours.

2.2. Bradford Assay

This assay is widely used to measure protein concentration in a sample using BSA as a substrate. Bradford reagent was prepared by mixing 0.01 g Coomassie brilliant blue (CBB) G-250 with 5 ml ethanol 95% (v/v) and 10 ml phosphoric acid 85% (v/v). The mixture was homogenized, filtered, and stored in dark bottle at low temperature. Standard protein solution was prepared by dissolve 0.01 gr BSA in 10 ml sterile water to have a stock solution with 1000 ppm. This solution was diluted by adding 0.5 ml stock solution with 4.5 ml sterile water to obtain 100 ppm solution. The solution was set for serial dilution at 0, 10, 20, 30, 40, 50, 60, 70, 80, 90, and 100 ppm. Protein standard was prepared by mixing 0.1 ml of serial dilution with 5 ml of Bradford reagent, vortexed, and measured at 595 nm [16]. The protein concentration was calculated using linear regression from the standard protein marker.

2.3. Sodium Dodecyl Sulphate Poly-Acrylamide Gel Electrophoresis (SDS-PAGE) Assay

This assay was carried out to separate the protein based on molecular mass, ranged between 1-100 kDa, and visualized using electrophoresis. This method using coomassie brilliant blue for staining. The technique uses 3% (wt/vol) polyacrylamide gel and strengthened with 0.5% (wt/vol) agarose. The band present on the gel showed the molecular mass of the protein.

3. RESULTS AND DISCUSSION

In this study, ES antigen of *Paramphistomum* spp. was evaluated. The results of BSA standard absorption showed that the regression equation was $Y = 0.451x +$

0.651, as presented in Figure 1. This standard curve is important to calculate the protein concentration of the sample, and the result showed that the absorbance in the sample ranged from 0.696-0.966. The absorbance value of BSA standard curve is listed in Table 1.

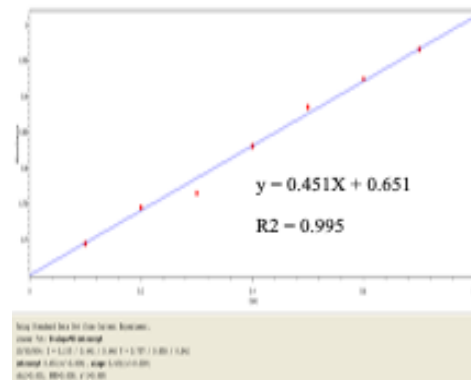


Figure 1 BSA solution standard curve

Table 1. Absorbance value of BSA standard curve

BSA concentration (mg/mL)	Absorbance
0.1	0.696
0.2	0.746
0.4	0.831
0.5	0.886
0.6	0.924
0.7	0.966

Based on Bradford method, the protein concentration from pellet and supernatant was calculated, and it was ranged between 66-261 ppm (Table 2).

Table 2. Protein concentration of excretory-secretory and somatic antigen

Treatment	Absorbance	Protein (ppm)
Supernatant 12 hours	0.715	142
Pellet 12 hours	0.681	66
Supernatant 24 hours	0.723	159
Pellet 24 hours	0.712	135
Supernatant 48 hours	0.757	235
Pellet 48 hours	0.769	261
Supernatant 72 hours	0.739	195
Pellet 72 hours	0.748	215

After 12 hours incubation, protein concentration in supernatant was higher (142 ppm) compared to pellet (66 ppm). After 24 hours, protein concentration in supernatant reached 159 ppm, while in pellet was 135 ppm. The highest concentration was obtained within 48 hours incubation, it was 235 ppm in supernatant, and 261 ppm in pellet. After 72 hours, the concentration was decreased both in pellet and supernatant. The high level of protein concentration in supernatant indicated the high level of ES antigen, while protein concentration in pellet indicated somatic antigen. Antigen which has major histocompatibility complex was owned by ES antigen with more specific protein structure. It is in accordance with study by Penn et al. [17] who conducted experiment with three trematode species. MHC is a group of genes which code for proteins found on the surface of cells that help the immune systems recognize foreign substances [18].

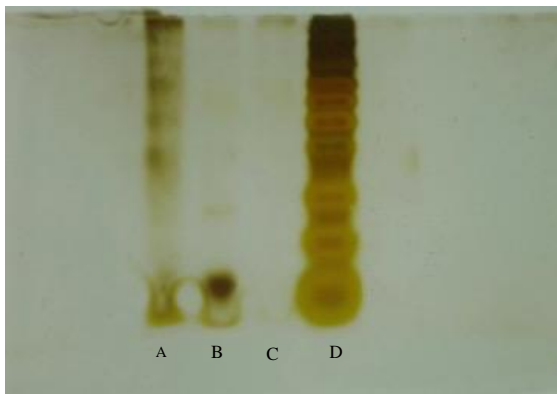


Figure 2 Visualization of protein bands in SDS-PAGE gel electrophoresis. A. 48 hours. B. 72 hours. C. 24 hours. D. Marker.

From SDS-PAGE result, it showed that the band of protein from ES antigen at 48-hour incubation was clearly seen, with molecular weight was ranged between 29-130 kDa. The band of protein at 24- and 72-hour incubation was degraded. The visualization of protein was presented in Figure 2. This figure points out that ES antigen of *Paramphistomum spp.* was present in the supernatant, and the media used in this experiment exhibited effectiveness for ES antigen isolation.

4. CONCLUSION

From the results it can be concluded that RPMI 1640 is a good choice for isolation of ES antigen from *Paramphistomum spp.* The optimum time for harvesting protein of ES antigen was 48 hours, and the concentration decreased over time. Molecular weight of ES antigen obtained was 29-130 kDa.

AUTHORS' CONTRIBUTIONS

The research was designed by MH and UB. The field work was conducted by HV and SRA. The manuscript was written by MH, HV, and SRA.

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